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Title: Contribution of oxidative stress to non-AIDS events in HIV-infected patients

Authors: Mar Masiá[1§], Sergio Padilla[1], Marta Fernández[2], Xavier Barber[3], Santiago Moreno[4], José Antonio Iribarren[5], Joaquín Portilla[6], Alejandro Peña[7], Francesc Vidal[8], Félix Gutiérrez[1], CoRIS.

Affiliations:

[1]Infectious Diseases Unit, Hospital General de Elche, Universidad Miguel Hernández, Alicante, Spain

[2]Infectious Diseases Research Lab, Hospital General de Elche, Spain

[3]Statistics, Centro de Investigación Operativa, Universidad Miguel Hernández, Elche, Alicante, Spain

[4]Infectious Diseases Service. Hospital Universitario Ramón y Cajal, IRYCIS, Madrid, Spain

[5]Infectious Diseases Unit. Hospital Universitario de Donostia, San Sebastián, Spain

[6] Infectious Diseases Unit. Hospital General Universitario de Alicante, Alicante, Spain

[7] Infectious Diseases Unit. Hospital Universitario San Cecilio, Granada, Spain

[8] Infectious Diseases Unit . Hospital Universitari de Tarragona Joan XXIII, IISPV, Universitat Rovira i Virgili, Tarragona, Spain

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[§]Corresponding author: Mar Masiá, MD, PhD. Hospital General Universitario de Elche, Universidad Miguel Hernández, Alicante, Spain. Phone: 00-34-96-667 91 54, Fax: 00-34-96-667 91 56, E-mail: marmasiac@gmail.com

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ABSTRACT

Objective: Recognition of potentially modifiable mechanisms implicated in the pathogenesis of non-AIDS events (NAEs) might help improve outcomes of HIV-infected individuals. HIV infection has been associated with increased oxidative stress. We assessed the association between F2-isoprostanes and serious NAEs, and whether they improve the predictive performance of inflammation and coagulation biomarkers.

Methods: Prospective multicenter cohort. Individuals who had an incident serious NAE and two sex and age matched participants with no events were selected. Measurement of F2-isoprostanes, highly-sensitive C-reactive protein (hsCRP), interleukin-6, D-dimer, sCD14, sCD40, sCD163 and neopterin levels was performed in successive plasma samples collected from cohort inclusion.

Results: Biomarkers were measured in 78 participants developing serious NAEs or death, and 151 subjects with no events. Adjusted levels of F2-isoprostanes, and also of hsCRP, sCD14 and D-dimer were higher in individuals who developed serious NAEs, including or not non-AIDS deaths. The same results were observed when only samples

collected since the time of achieving virological suppression were analyzed. The additive incorporation of each biomarker, ending with F2-isoprostanes, in an adjusted model was associated with a graded and significant increase in the quality of model fitting, and 94% sensitivity, 33% specificity and 0.77 accuracy to predict serious NAEs including non-AIDS-related death.

Conclusion: Oxidative stress is associated with a higher risk of serious NAEs, including non-AIDS deaths. This effect is independent and additive to biomarkers of inflammation, monocyte activation and coagulation. Our results suggest that oxidative stress should be included among mechanisms to deal with to improve prognosis of HIV-infected individuals.

Keywords: HIV pathogenesis; pro-oxidant status; reactive oxygen species; prognosis; non-routine biomarkers; intervention

INTRODUCTION

Non-AIDS events have become major causes of morbidity and mortality in people living with HIV (PLWH) in high income countries since the introduction of effective antiretroviral therapy (ART).[1,2] Given that mortality in treated PLWH remains higher than in the general population,[3] the mechanisms involved in the pathogenesis of non-AIDS events have gained attention in recent years. Chronic inflammation and immune activation have demonstrated to play a prominent role in the pathogenesis of HIV disease.[4] Several studies support their association with HIV disease progression, and to persist despite effective ART.[5,6] Elevated levels of biomarkers of inflammation, immune activation and coagulation have also been related to the development of non-AIDS

events, mostly in single measurements performed in patients who had not yet started ART,[7-11] although data also exist with longitudinal samples from virologically suppressed people on ART.[12] Interventional strategies targeting factors associated with inflammation and immune activation in individuals receiving ART have shown dissimilar degrees of success and little clinical benefit up to now, except for the treatment of hepatitis C virus (HCV) coinfection.[13,14] Additional or simultaneous interventions and/or the recognition of further potentially modifiable mechanisms implicated might help to improve outcomes of PLWH.

HIV infection is associated with a pro-oxidative status due to the imbalance between the generation of reactive oxygen species and the antioxidant capacity of the organism.[15] Oxidative stress has been implicated in many aspects of HIV pathogenesis, including stimulation of HIV replication, numerical and functional impairment of $CD4^+$ T cells, altered immune response, and antiretroviral drug toxicity.[16-20] Enhanced oxidative stress is involved in aging and probably in the pathogenesis of certain clinical disorders in the general population[21-23] but, to the best of our knowledge, the contribution of oxidative stress to the development of serious non-AIDS events has not yet been explored.

Measurement of F2-isoprostanes constitutes the most reliable approach to assess oxidative stress status in vivo.[24] We evaluated here the association of successive determinations of F2-isoprostanes, as well as of biomarkers of inflammation, monocyte activation and coagulation, with the risk of severe non-AIDS comorbidities, including death, in PLWH from the time of engagement in care in a multicenter cohort. We also

developed predictive models for the occurrence of non-AIDS events by the sequential addition of the biomarkers.

METHODS

Design and study subjects

The study was conducted in the ongoing open cohort of adults with HIV infection of the Spanish AIDS Research Network (CoRIS). This is a prospective, multicentre cohort of adult subjects with confirmed HIV infection, and naïve to ART at study entry. The cohort is linked to a centralized BioBank, where blood samples are processed, cryopreserved and stored. A baseline sample is obtained at cohort entry, and follow-up samples are collected annually or biannually thereafter. The BioBank has obtained the UNE-EN-ISO 9001:2008 Systems of Quality Management Requirements. Approval from each hospital's Ethics Committee, and written informed consents from the patients, including the specific consent for the BioBank, were obtained. Detailed description of CoRIS and the BioBank have been previously reported.[25,26]

All centers were invited in February 2008 to report all non-AIDS events occurring from the day of entry in the cohort.[27] All deaths occurring from the day of inclusion in the cohort not attributed to AIDS causes (non-AIDS deaths) were also included in the analyses of non-AIDS events as secondary outcome variable. Centers were provided with a structured event reporting form containing the list of events to be reported and the precise definition of each non-AIDS event required for the inclusion (see Supplemental Data Files, http://links.lww.com/ QAD/A261). Investigators also had to fill a specific event form for each particular non-AIDS event with additional data

detailing the event. Death due to a non-AIDS event was classified according to a revised version of the 'Coding Death in HIV' (CoDe) classification system (http://www.cphiv.dk).

For the purpose of this study, we selected all incident serious non-AIDS events, including the following: cardiovascular-related (acute myocardial infarction, angina, congestive heart failure, stroke, transient ischemic attack, silent cerebrovascular disease, peripheral arterial disease, coronary-related death), non-AIDS-defining malignancies, renal-related (acute renal failure, chronic kidney disease, renal tubulopathy/Fanconi syndrome, permanent dialysis, kidney biopsy), and liver-related (ascites, hepatic encephalopathy, variceal hemorrhage, hepatic transplant, hepatocellular carcinoma, liver insufficiency/cirrhosis).

The study population included all volunteers with available blood samples at the BioBank from cohort launching date (January 01, 2004) to administrative censoring date (October 31, 2010). We selected individuals who had an incident serious non-AIDS event during the study period, and a group of two sex and age matched participants per case that entered the cohort during the same study period and who did not develop non-AIDS events. Based on previously published data,[7] for 78 individuals who developed serious incident non-AIDS events or death and had blood samples at the Biobank, we selected 151 non-AIDS event or death-free matched individuals. With this sample we estimated a statistical power of 80% to detect a difference of at least 40% between groups, assuming a 15% of pre-analytical sample losses. Blood samples were kindly provided by the BioBank. All available samples for each individual were analysed.

Plasma biomarkers determinations

Plasma aliquots obtained were stored at -80ºC. All frozen samples were subsequently defrosted for their analysis. Commercially available ELISA kits were used to measure plasma levels of sCD14 (Quantikine, R&D Systems Europe Ltd, UK), interleukin-6 (IL-6), sCD40, sCD163 (DuoSet, R&D Systems Europe Ltd, UK), D-dimer (Technozym® D-Dimer ELISA, Technoclone GmbH, Vienna/Austria), neopterin (IBL International GmbH, Hamburg/Germany) and F2-isoprostanes (Cayman Chemical Company, Michigan/USA). Highly-sensitive C-reactive protein (hsCRP) was measured with a chemiluminescent immunometric assay (Immulite® 2000 autoanalyzer, Siemens, Germany).

Statistical analyses

Statistical analyses of the data were performed in R, version 3.0.2 (R Foundation for Statistical Computing, Vienna, Austria, URL http://www.R-project.org/). Median values were compared with the Mann-Whitney or Wilcoxon tests, where appropriate, and chi-square was used to compare proportions. Biomarkers values were logarithmically transformed. Since data were collected longitudinally, we used generalized additive mixed models (GAMM) analysis to study the associations of baseline and follow-up values of biomarkers with non-AIDS events and non-AIDS mortality, which allowed an appropriate statistical management of repeated longitudinal measurements of the same participant. Effects were quantified in terms of the difference in $\log_{10}$-tranformed biomarker level between patients with and without non-AIDS events. Adjusted analyses controlled for age, sex, CD4 cell count, HIV RNA and HCV coinfection were carried out. Two models adjusted for sex, age at cohort entry, RNA-HIV viral load and CD4-T cell count closest to biomarker determination, and HCV coinfection were developed to evaluate additive contribution of each biomarker to improve the predictive ability of the models. Main

outcome variable was serious non-AIDS event development, and secondary outcome variable was serious non-AIDS event development including non-AIDS deaths.

RESULTS

A total of 78 individuals with available blood samples developed serious incident non-AIDS events or death during follow-up. One hundred twelve serious non-AIDS events according to the definition mentioned above occurred in 64 subjects, and comprised 32 (28.6%) liver (19 decompensated cirrhosis), 28 (25%) renal (acute kidney failure 16, chronic renal failure 12), 26 (23.2%) malignant (24 non-metastatic neoplasms) and 26 (23.2%) cardiovascular events (13 acute myocardial infarction). A further 14 subjects died due to non-AIDS causes. Two hundred thirty two individuals without available blood samples in the Biobank developed serious non-AIDS events or non-AIDS death. There were no significant differences between participants with or without available blood samples in age, sex, CD4 cell count and HIV RNA at cohort entry, and in CD4 cell nadir (data not shown). One hundred fifty one sex and age matched individuals among those with available blood samples at the Biobank and without events were selected for comparison.

Baseline characteristics of the participants are shown in Table 1. Median follow-up (interquartile range, IQR) was 5.06 (2.98-6.87) years, with no differences between volunteers who developed or not events, nor were there differences between groups in the median HIV RNA levels at cohort entry. Participants developing non-AIDS events excluding death were more frequently Spanish (84.4% vs 67.3%, P=0.013) and injecting drug users (IDUs) (26.5% vs. 10.6%, P=0.011) than those who did not, and had less

frequently higher education (9.3% vs 24.7%, P=0.008), and had lower baseline CD4 cell counts (210 [54-439] vs 313 [149-511], P=0.030). The proportion of individuals with virological suppression in the last available sample for biomarkers measurement was 64.7 %, with no differences between groups, and median (IQR) CD4 cell count was 463 (283-650), with lower values observed in participants developing non-AIDS events (Table 1).

Biomarkers of oxidative stress

Results of the successive measurements of the biomarkers according to the occurrence of non-AIDS events are shown in Figure 1a. Median (IQR) number of determinations was 2 (1-3) per patient. Plasma levels of F2-isoprostanes were higher in subjects who developed incident non-AIDS events, including and not including non-AIDS deaths (Figure 1b). After adjustment for sex, age, HIV RNA, CD4-T cell count and HCV coinfection, higher levels of F2-isoprostanes were still associated with non-AIDS events occurrence, including or not non-AIDS deaths (Table 2).

Plasma samples collected since the time of achieving virological suppression (HIV RNA < 200 copies/ml) were selected for analysis. Adjusted levels of F2-isoprostanes were again significantly higher in subjects with non-AIDS events occurrence including or not non-AIDS deaths (Table 2)

Biomarkers of inflammation, monocyte activation and coagulation

Plasma levels of the inflammation biomarkers hsCRP, the monocyte activation biomarker sCD14, and of D-dimer were also higher in participants with incident non-AIDS events (Figures 1a and 1b). After adjustment, the association of the above mentioned biomarkers remained significant, and the same occurred when non-AIDS events or non-AIDS mortality was selected as endpoint measured (Table 2).

The analysis of samples collected since the time of achieving virological suppression showed again a significant association of non-AIDS events with higher levels of hsCRP, sCD14 and D-dimer in the adjusted analysis (Table 2), including or not including non-AIDS deaths.

Combination of biomarkers and risk of developing serious non-AIDS events

Two models adjusted for demographic and immunovirologic factors were developed to evaluate whether the sequential addition of each biomarker improved the accuracy of the model to predict non-AIDS events development (Table 3). The outcome variable was serious non-AIDS events occurrence in both models, and one of them included also death due to non-AIDS causes. The additive inclusion to a model initially containing hsCRP of D-dimer, sCD14 and finally F2-isoprostanes, significantly improved the deviance in the model including non-AIDS death. The final model including all biomarkers had a sensitivity of 94%, specificity of 33%, and an accuracy of 0.77 (95% CI, 0.73-0.81, P=0.0003), to predict the development of serious non-AIDS events including non-AIDS death, and a sensitivity of 88%, specificity of 56% and accuracy of

0.76 (95% CI, 0.71-0.81) to predict serious non-AIDS events excluding non-AIDS death.

DISCUSSION

In this study carried out in a contemporary cohort, higher levels of biomarkers of oxidative stress were associated with a higher risk of serious non-AIDS events. This association was independent of demographic, HCV and HIV-related factors and, importantly, remained significant when only samples of patients virologically suppressed with ART were analyzed. Our study also shows that the association of oxidative stress with non-AIDS events was independent of inflammation, monocyte activation and coagulation biomarkers, as shown in Table 3; moreover, the addition of biomarkers of oxidative stress to an adjusted predictive model containing the above mentioned biomarkers improved the model's predictive performance.

To the best of our knowledge, the role of oxidative stress to predict the development of non-AIDS events in HIV-infected individuals had not previously been described. HIV infection induces, both directly and indirectly, the generation and accumulation of reactive oxygen species (ROS), leading to a pro-oxidative status and oxidative DNA damage.[16,19,28] ART has also been associated with increased oxidative stress, although studies were mostly conducted in the early ART era,[17,20] and a protective effect of ART has also been described.[19] In addition to HIV infection, several factors may contribute to increase oxidative stress both in the HIV-infected and uninfected population. Certain RNA and DNA viruses, such as cytomegalovirus, influenza, and hepatitis A, B and C, can result in increased ROS production and oxidative stress,[29-31] as well as an intensive

physical activity, or the action of pollutants/toxins such as cigarette smoke, alcohol, ionizing and UV radiations and pesticides.[32]

Apart from the implication in the pathogenesis of HIV disease and the progression from the asymptomatic stage to the development of AIDS, the pro-oxidant status might contribute to the development of comorbid diseases within this population. Overproduction of ROS may lead to damage of cell structures, including lipids and membranes, proteins, and DNA. In the general population it has been implicated in various pathological conditions involving cardiovascular disease, cancer, neurological disorders, diabetes, ischemia/reperfusion, and in the aging process.[33] Reactive oxygen species cause damage to mitochondrial components and initiate degradative processes that contribute significantly to aging.[34] Persistent oxidative stress has shown to accelerate telomere attrition and the loss of telomerase activity, leading to the disruption of telomere integrity and the consequent triggering of premature senescence.[35,36] Telomere dysfunction and the associated premature senescence of endothelial cells have been implicated in the pathogenesis of vascular disease.[35] The redox imbalance and the ROS-induced DNA damage have been also associated with mutagenesis and carcinogenesis, and mitochondrial oxidative stress has been linked with diabetes mellitus.[33] We recently found that a single baseline measurement of F2-isoprostanes was associated with all-cause mortality in PLWH.[37] The present study shows that oxidative stress is also a strong predictor for the occurrence of comorbid diseases, and this predictive ability is independent of the immunologic and virologic status of the individuals, with a persistent predictive performance in virologically suppressed persons.

We assessed the additional contribution of oxidative stress biomarkers in relation to other non-routine biomarkers to predict the prognosis of PLWH. We found that the sequential addition of biomarkers of inflammation, monocyte activation, coagulation and oxidative stress in an adjusted model to predict the occurrence of serious non-AIDS events including non-AIDS death, improved the quality of model-fitting. Therefore, adding successively D-dimer, sCD14 and, finally, F2-isoprostanes to a model initially including hsCRP levels, significantly and gradually enhanced the predictive performance of the model, with a high sensitivity and good accuracy. Accordingly, clustering of elevated levels of the biomarkers in an individual person is associated with improved risk prediction of comorbidity or death occurrence compared to that of one isolated biomarker. Another implication of our findings is that, while probably interrelated, these biomarkers designate independent pathogenic pathways, and interventions aiming at reducing morbidity and mortality in PLWH should ideally take all of them into account. Because of the added prognostic power shown in our study, oxidative stress should be included among the mechanisms to deal with to improve health and survival of PLWH, and to check in order to assess individuals' likelihood of comorbidity development. As a consequence, antimicrobial therapy directed at microorganisms implicated in oxidative stress, such as cytomegalovirus or viral hepatitis, as well as replenishment with antioxidants, like selenium, zinc, or antioxidant compounds like N-acetyl cysteine or glutathione,[29,31] might also be considered as potential measures to reduce the pro-oxidant status and consequently to prevent comorbidity development.[38]

Few studies have evaluated to date the role of successive measurements of different biomarkers as predictors of non-AIDS events occurrence. We found that biomarkers were independently associated with increased risk of serious events occurrence even

when fatal cases were excluded, although the magnitude of the association was lower in that case, suggesting a positive relationship between the predictive performance and event severity. Alternatively, the reduction in sample size might have also affected the statistical power. Elevated biomarkers of inflammation and coagulation have been associated with mortality,[39-41] and less frequently with non-AIDS events development, mostly in cross-sectional studies.[7-11] Availability of sequential levels of the biomarkers gives consistency to our findings, because the association with events is established with more than one single measurement. In addition, it supports their predictive role at different times in the course of disease, under diverse immunologic and virologic conditions. Noteworthy, the predictive ability of the biomarkers persisted when only samples from patients with virological suppression were analysed, supporting that ART does not completely control these pathogenic pathways. Our results are in agreement with those of Tenorio et al.,[12] who found that serial measurements of inflammation and coagulation markers predicted non-AIDS events occurrence. That study included HIV-infected subjects who had achieved virological suppression within 1 year after ART initiation, and blood samples were obtained at 3 time-points: before ART initiation, 1-year after ART initiation, and at the immediate visit preceding the event.[12] In our cohort, higher levels of sCD14, a biomarker associated with monocyte activation and bacterial translocation,[42] were also associated with the development of non-AIDS events. Among the differences with the study by Tenorio et al., our study also included different event categories, such as liver and kidney-related, and did not include bacterial infections. which could likewise explain differences in some results between both studies.

A limitation of the study was the unavailability of information about traditional risk factors for non-AIDS events, like smoking or lifestyle habits, to be included in adjustments. The small sample size, especially for some sub-analyses, as well as the differences in some characteristics at baseline between groups, which could have confounded the analysis, also constitute limitations of the study. A high number of individuals with serious non-AIDS events/non-AIDS death had no available blood samples to be included in the analysis; however, they were not significantly different from those with available samples at the Biobank. The model had good sensitivity but low specificity; as a consequence, preventive measures would be implemented in many non-high-risk PLWH if the model was used with that purpose. Strengths are the availability of successive blood samples and the large number of biomarkers analysed, reflecting several and non-explored pathogenic pathways.

In conclusion, increased oxidative stress is associated with the occurrence of serious non-AIDS events. This effect is independent of the virologic and immunologic status of the patients, and of the presence of other non-routine prognostic biomarkers. Our results suggest that oxidative stress should be included among mechanisms to deal with to improve outcomes of PLWH. Combination of oxidative stress with biomarkers of inflammation, monocyte activation and coagulation improves prediction of serious non-AIDS events. Further studies are needed to define the optimal cut-off points that might potentially help clinicians in the management of PLWH.

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Centers and investigators participating in CoRIS, Biobanco:

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Field work, data Management and analysis: Paz Sobrino Vegas, Victoria Hernando Sebastián, Belén Alejos Ferreras, Débora Álvarez, Susana Monge, Inmaculada Jarrín, Adela Castelló.

BioBanco: M Ángeles Muñoz-Fernández, Isabel García-Merino, Coral Gómez Rico, Jorge Gallego de la Fuente y Almudena García Torre.

Participating centers:

Hospital General Universitario de Alicante (Alicante): Joaquín Portilla Sogorb, Esperanza Merino de Lucas, Sergio Reus Bañuls, Vicente Boix Martínez, Livia Giner Oncina, Carmen Gadea Pastor, Irene Portilla Tamarit, Patricia Arcaina Toledo.

Hospital Universitario de Canarias (Santa Cruz de Tenerife): Juan Luis Gómez Sirvent, Patricia Rodríguez Fortúnez, María Remedios Alemán Valls, María del Mar Alonso Socas, Ana María López Lirola, María Inmaculada Hernández Hernández, Felicitas Díaz-Flores.

Hospital Carlos III (Madrid): Vicente Soriano, Pablo Labarga, Pablo Barreiro, Pablo Rivas, Francisco Blanco, Luz Martín Carbonero, Eugenia Vispo, Carmen Solera.
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Hospital General Universitario de Elche (Elche): Félix Gutiérrez, Mar Masiá, José Manuel Ramos, Sergio Padilla, Andrés Navarro, Fernando Montolio, Yolanda Peral, Catalina Robledano, Joan Gregori
Hospital Germans Trías i Pujol (Badalona): Bonaventura Clotet, Cristina Tural, Lidia Ruiz, Cristina Miranda, Roberto Muga, Jordi Tor, Arantza Sanvisens
Hospital General Universitario Gregorio Marañón (Madrid): Juan Berenguer, Juan Carlos López Bernaldo de Quirós, Pilar Miralles, Jaime Cosín Ochaíta, Isabel Gutiérrez Cuellar, Margarita Ramírez Schacke, Belén Padilla Ortega, Paloma Gijón Vidaurreta, Ana Carrero Gras, Teresa Aldamiz-Echevarría Lois y Francisco Tejerina Picado.
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Hospital Universitario La Fe (Valencia): José López Aldeguer, Marino Blanes Juliá, José Lacruz Rodrigo, Miguel Salavert, Marta Montero, Eva Calabuig, Sandra Cuéllar.
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Hospital San Pedro II (Logroño): Javier Pinilla Moraza
Hospital de Navarra (Pamplona): Julio Sola Boneta, Javier Uriz, Jesús Castiello, Jesús Reparaz, María Jesús Arraiza, Carmen Irigoyen, David Mozas,
Hospital Ramón y Cajal (Madrid): Santiago Moreno, José Luis Casado, Fernando Dronda, Ana Moreno, María Jesús Pérez Elías, Dolores López, Carolina Gutiérrez, Beatriz Hernández, María Pumares, Paloma Martí.
Hospital Reina Sofía (Murcia): Alfredo Cano Sánchez, Enrique Bernal Morell, Ángeles Muñoz Pérez
Hospital San Cecilio (Granada): Federico García García, José Hernández Quero, Alejandro Peña Monje, Leopoldo Muñoz Medina, Jorge Parra Ruiz.
Centro Sanitario Sandoval (Madrid): Jorge Del Romero Guerrero, Carmen Rodríguez Martín, Teresa Puerta López, Juan Carlos Carrió Montiel, Cristina González, Mar Vera.
Hospital Universitario Santiago de Compostela (Santiago de Compostela): Antonio Antela, Arturo Prieto, Elena Losada
Hospital Son Espases (Palma de Mallorca): Melchor Riera, Javier Murillas, Maria Peñaranda, Maria Leyes, Mª Angels Ribas, Antoni Campins, Concepcion Villalonga, Carmen Vidal.
Hospital Universitario de Valme (Sevilla): Juan Antonio Pineda, Eva Recio Sánchez, Fernando Lozano de León, Juan Macías, José del Valle, Jesús Gómez-Mateos, Rosario Mata.
Hospital Virgen de la Victoria (Málaga): Jesús Santos González, Manuel Márquez Solero, Isabel Viciana Ramos, Rosario Palacios Muñoz
Hospital Universitario Virgen del Rocío (Sevilla): Pompeyo Viciana, Manuel Leal, Luis Fernando López-Cortés, Mónica Trastoy.

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Figure 1. Biomarkers values obtained on successive measurements in patients with and without non-AIDS events (panel a, patients who developed non-AIDS events and patients without events; panel b, patients who developed non-AIDS events or died and patients without events). The central line represents the median.

Table 1.Baseline characteristics of study patients.

	Serious NAEs			Serious NAEs or death		
	No	**Yes**	**p¶**	**No**	**Yes**	**p¶**
Patients, no.	113	64	-	151	78	-
Female, no. (%)	19 (16.8)	11 (17.1)	0.949	21 (13.9)	12 (15.3)	0.762
Age at cohort entry, median years (IQR)	42.4 (34.8-49.9)	44.2 (38.1-51.6)	0.086	43.5 (35.0-51.5)	44.5 (38.1-53.0)	0.417
HIV transmission groups, no. (%)						
IDU	12 (10.6)	17 (26.5)	0.011	19 (12.6)	18 (23.0)	0.041
Non-IDU	101 (89.4)	47 (73.4)		132 (87.4)	60 (76.9)	
Educational level*			0.008			0.002
Low	43 (38.0)	40 (62.5)		56 (37.1)	49 (62.8)	
Medium	34 (30.0)	13 (20.3)		44 (29.1)	14 (17.9)	
High	28 (24.7)	6 (9.3)		33 (21.9)	8 (10.2)	
Unknown	8 (7.0)	5 (7.8)		18 (11.9)	7 (8.9)	
Country of origin			0.013			0.008
Spain	76 (67.3)	54 (84.4)		101 (66.9)	65 (83.3)	
Other	37 (32.7)	10 (15.6)		50 (33.1)	13 (16.7)	
CD4 (cells/µL) at cohort entry, no. (IQR)	313 (149-511)	210 (54-439)	0.030	321 (180-511)	205 (58-471)	0.022
CD4 (cells/µL) at final visit, no. (IQR)	518 (319-664)	399 (234-624)	0.014	517 (319-656)	300 (128-553)	0.004
Plasma HIV viral load (log10, copies/mL) at cohort entry, median (IQR)	4.57 (3.98-507)	4.77 (3.90-5.36)	0.527	4.55 (3.99-5.06)	4.79 (3.9-5.3)	0.260
Patients with HIV-RNA < 200 copies/mL at final visit, (%)	72 (63.3)	43 (67.1)	0.731	104 (69.2)	44 (56.5)	0.184
Hepatitis C virus coinfection, no. (%)	23 (20.3)	21 (32.8)	0.096	30 (19.8)	24 (30.7)	0.065
Follow-up¥, median years (IQR)	5.19 (2.91-7.07)	5.00 (3.38-7.14)	0.500	5.03 (2.91-2.17)	4.63 (2.52-5.96)	0.209
Number of samples per patient, median (min, Q1, Q3,max); mean	2 (1,1,3,3)	1 (1,1,3,3); 1.81	0.898	2 (1,1,3,3)	1 (1,1,3,3); 1.83	0.685

NAEs, non-AIDS events; IQR, interquartile range; IDU, injection drug users. ¶, *p* value between patients with and without events; * Educational level was defined based on the level of education reached at cohort entry, and subjects were classified into three levels: low, individuals with no education or with primary education; medium, individuals who completed secondary education; and high, individuals who completed university education. ¥, Years from cohort inclusion to which happened first: death, lost of follow-up or administrative censoring.

Table 2. Absolute log_{10} difference in the levels of the biomarkers between patients who developed non-AIDS events and patients who did not, according to the virological status of the patients. Results are also shown for patients who developed non-AIDS events or death

Biomarker	Unadjusted analysis				Adjusted analysis *			
	All samples		Samples collected during virological suppression		All samples		Samples collected during virological suppression	
	Absolute log_{10} difference between biomarkers (standard error)	p	Absolute log_{10} difference between biomarkers (standard error).	p	Absolute log_{10} difference between biomarkers (standard error).	p	Absolute log_{10} difference between biomarkers (standard error).	p
Serious NAE (n=64 patients#):								
hsC-reactive protein (mg/dL)	+0.56 (0.16)	0.001	+0.61 (0.21)	0.004	+0.25 (0.07)	0.001	+0.26 (0.09)	0.004
IL-6 (pg/mL)	+0.36 (0.20)	0.070	+0.17 (0.29)	0.553	+0.13 (0.08)	0.116	+0.08 (0.12)	0.517
Neopterin (nmol/L)	+0.24 (0.12)	0.052	+0.20 (0.18)	0.267	+0.11 (0.05)	0.026	+0.06 (0.07)	0.412
Free-F2-isoprostanes (pg/mL)	+0.19 (0.09)	0.037	+0.13 (0.14)	0.358	+0.09 (0.04)	0.019	+0.06 (0.04)	0.048
D-dimer (ng/mL)	+0.44 (0.25)	0.087	+0.65 (0.33)	0.052	+0.22 (0.10)	0.036	+0.33 (0.14)	0.025
s-CD14 (pg/mL)	+0.17 (0.05)	0.001	+0.12 (0.07)	0.080	+0.06 (0.02)	0.004	+0.05 (0.02)	0.084
s-CD163 (ng/mL)	+0.17 (0.17)	0.316	+0.19 (0.23)	0.412	+0.07 (0.07)	0.341	+0.03 (0.09)	0.739
s-CD40 (pg/mL)	-0.13 (0.25)	0.609	-0.09 (0.39)	0.803	-0.04 (0.11)	0.697	-0.03 (0.16)	0.824
Serious NAE or Death (n=78 patients¥):								
hsC-reactive protein (mg/dL)	+0.67 (0.13)	0.001	+0.69 (0.18)	0.001	+0.28 (0.05)	0.001	+0.30 (0.08)	0.001
IL-6 (pg/mL)	+0.66 (0.18)	0.001	+0.44 (0.26)	0.094	+0.28 (0.08)	0.001	+0.11 (0.11)	0.329
Neopterin (nmol/L)	+0.18 (0.11)	0.088	+0.16 (0.15)	0.285	+0.09 (0.04)	0.049	+0.06 (0.06)	0.324
Free-F2-isoprostanes (pg/mL)	+0.28 (0.08)	0.001	+0.23 (0.12)	0.058	+0.12 (0.03)	0.001	+0.09 (0.05)	0.035
D-dimer (ng/mL)	+0.90 (0.23)	0.001	1.26 (0.30)	0.001	+0.42 (0.09)	0.001	+0.51 (0.13)	0.001
s-CD14 (pg/mL)	+0.21 (0.04)	0.001	+0.15 (0.06)	0.010	+0.07 (0.01)	0.001	+0.05 (0.02)	0.028
s-CD163 (ng/mL)	+0.14 (0.13)	0.280	+0.28 (0.17)	0.116	+0.05 (0.05)	0.325	+0.03 (0.07)	0.694
s-CD40 (pg/mL)	+0.04 (0.20)	0.826	-0.16 (0.30)	0.595	+0.06 (0.08)	0.495	-0.07 (0.12)	0.566

NAE, non-AIDS event; *, Adjusted for sex, age, RNA-HIV and CD4-T cell count and hepatitis C virus coinfection. #, The number of serum samples tested were 321 and 151 for all patients and for the sub-group of patients with virological suppression, respectively. ¥, The number of serum samples tested were 511 and 228 for all patients and for the sub-group of patients with virological suppression sub-group, respectively. hsCRP, highly sensitive C-reactive protein; IL-6, interleukin-6

Table 3. Adjusted model showing the association of the sequential addition of each biomarker with the occurrence of non-AIDS events, including and not including death

			All samples		Model performance				
Outcome variable	**Model #**	**Variables included in Model***	**Deviance explained (%)**	***p* value**	***Accuracy (95% CI)***	***Sen.***	***Spe.***	***PPV***	***NPV***
Serious NAE	**1**	hsCRP	16.5	-	0.74 (0.68-0.78)	0.92	0.42	0.73	0.75
	2	Model#1 + IL-6	16.6	0.451	0.75 (0.69-0.79)	0.91	0.46	0.75	0.74
	3	Model#2 + D-Dimer	17.1	0.103	0.74 (0.69-0.79)	0.90	0.46	0.75	0.72
	4	Model#3 + s-CD14	17.4	0.307	0.73 (0.68-0.78)	0.88	0.47	0.75	0.70
	5	Model#4 + F2-isoprostanes	28.7	0.001	0.76 (0.71-0.81)	0.88	0.56	0.78	0.72
Combined outcome: Serious NAE or Non-AIDS condition related-Death	**1**	hsCRP	11.5	-	0.75 (0.70-0.78)	0.96	0.20	0.75	0.69
	2	Model#1 + IL-6	13.4	0.048	0.75 (0.71-0.79)	0.95	0.25	0.76	0.68
	3	Model#2 + D-Dimer	15.7	0.001	0.75 (0.71-0.79)	0.94	0.28	0.77	0.65
	4	Model#3 + s-CD14	16.0	0.104	0.76 (0.72-0.79)	0.94	0.29	0.77	0.66
	5	Model#4 + F2-isoprostanes	19.3	0.005	0.77 (0.73-0.81)	0.94	0.33	0.78	0.71

*All models also included sex, age at cohort entry, RNA-HIV viral load and CD4-T cell count closest to biomarker determination and hepatitis C virus coinfection. P value refers to the p value of ANOVA test for difference between nested models. Accur., accuracy (95% CI); Sen., sensitivity; Spe., specificity; PPV, positive predictive value; NPV, negative predictive value; NAE, non-AIDS event; hsCRP, highly sensitive C-reactive protein; IL-6, interleukin-6